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The latex obtained from *Hancornia speciosa* Gomes possesses anti-inflammatory activity

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ABSTRACT

Aim of the study: Hancornia speciosa Gomes (Apocynaceae) is a tree that is widely distributed throughout Brazil. Its latex is collected and used extensively to treat acne, warts, diseases related to bursitis, and inflammation. In this work, we describe the anti-inflammatory effects of the latex.

Materials and methods: The latex from *Hancornia speciosa* (0.06–1.3 mg/kg, p.o.) and the reference drug acetylsalicylic acid (ASA, 200 mg/kg, p.o.) were evaluated in analgesia (formalin-induced licking, acetic acid-induced contortions, and hot plate) and inflammation models (formalin-induced licking, paw oedema, and subcutaneous air pouch, with measurement of cell migration, exudate volume, protein extravasations, nitric oxide, prostaglandin E2, TNF-α, and IL-6, and expression of the enzymes inducible nitric oxide synthase and cyclooxygenase 2).

Results: The latex from *Hancornia speciosa* significantly inhibited the number of writhings and the time that the animal spent licking the formalin-injected paw (second phase). Doses of 0.1–1.3 mg/kg latex reduced carrageenan-induced rat paw oedema. However, only the highest doses (0.6 and/or 1.3 mg/kg) reduced the oedema induced by bradykinin, histamine, and serotonin. The latex also inhibited inflammation induced by subcutaneous carrageenan injection, cell migration, exudate volume, protein extravasations, increased levels of inflammatory mediators (nitric oxide, prostaglandin E2, TNF-α, and IL-6) produced in the pouch, and increased expression of the enzymes nitric oxide synthase and cyclooxygenase 2.

Conclusions: Our results indicate that the latex obtained from *Hancornia speciosa* demonstrates significant anti-inflammatory activity through the inhibition of nitric oxide, PGE2, and cytokine production, thus confirming the popular use of this plant as an anti-inflammatory agent.

1. Introduction

The Mangabeira, *Hancornia speciosa* Gomes (Apocynaceae), is a tree found naturally in Brazil. It is distributed throughout the Midwest, Southeast, North, and Northeast regions, with greater abundance in the coastal plains areas and tablelands of the Northeast. These areas are home to almost the entire national crop. In an ethnopharmacological study of the residents of the Caipé village (São Cristovão, Sergipe, Brazil), there is an extensive use of the latex obtained from the trunk to treat diseases related to fungal infection, tuberculosis, and ulcers. It is also used to stimulate hepatic function, to treat acne and warts, and to improve strains, dislocations and certain types of inflammation (Pott and Pott, 1994).

It has been reported that flavonoids, catechins, proanthocyanidines, and tannins are present in the bark of *Hancornia speciosa*; in addition, steroids, triterpenes, and tannins are present in the leaves (Moraes et al., 2008). Some pharmacological effects of *Hancornia speciosa* have also been demonstrated, such as an endothelium-dependent vasodilatory effect from the ethanol extract obtained from the leaves (Ferreira et al., 2007). Other effects ascribed to the extracts from the barks include gastroprotective, healing, and anti-*Helicobacter pylori* activities (Moraes et al., 2008). There have been a few articles that have reported pharmacological effects induced by the latex obtained from the trunk (the main product used in folk medicine). Recently, Silva et al. (2010) described the antifungal activity of the latex against *Candida albicans*.

Because of the intense popular use of the latex and the lack of studies analysing its medicinal effects, we endeavoured to evalu-

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ate the anti-inflammatory and antinociceptive effects of the latex obtained directly from the trunk of *Hancornia speciosa*.

2. Materials and methods

2.1. Plant material

The latex of *Hancornia speciosa* was collected by Celuta S. Alviano, in Abaís, Sergipe (Brazil). A voucher specimen was deposited at the Federal University of Sergipe Herbarium, Universidade Federal de Sergipe (Sergipe, Brazil); the exsiccate received the number ASE 13,630. The latex was collect by drilling in the tree trunk. The latex that dripped after drilling was collected in a sterile container with distilled water in a 2:1 (latex:water) proportion. After collection, the drilled area was sealed with inert and sterile wax to prevent further contamination of the tree.

2.2. Protein quantification of the latex

The *Hancornia speciosa* latex protein content was quantified using Lowry's colorimetric assay (Lowry et al., 1951).

2.3. Animals

All experiments were performed with male BALB/c mice (20–25 g) or Wistar rats (150–200 g) obtained from our own animal facility. Animals were maintained in a room with controlled temperature ($22 \pm 2\,^{\circ}C$) with a 12-h light/dark cycle and free access to food and water. Twelve hours before each experiment, the animals received only water to prevent the food from interfering with absorption of the substances. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA). They were approved by the Ethical Committee for Animal Research (Biomedical Science Institute/UFRJ) and received the number ICBDFBC-015.

2.4. General

Acetylsalicylic acid (ASA), carrageenan, bradykinin, serotonin, and histamine were purchased from Sigma–Aldrich (St. Louis, MO, USA), and acetic acid was purchased from Merck (Rio de Janeiro, Brazil). Nitrocellulose membranes (0.25 μm) were from Bio-Rad Laboratories, anti-mouse iNOS and COX2 antibodies were from Santa Cruz Biotechnology and anti-mouse IgG antibody conjugated to horseradish peroxidase and enhanced chemiluminescence (ECL) kits were from Amersham. All drugs were dissolved in phosphate buffered saline (PBS) just before use. Latex was dissolved in sterile water and administered by oral gavage at doses of 0.06–1.3 mg of protein/kg in a final volume 0.1 ml. ASA (200 mg/kg) was used as a reference drug and was administered by oral gavage. The negative control group was composed of animals treated with vehicle.

2.5. Acute toxicity

The parameters were determined as described by Lorke (1983). A single oral dose of *Hancornia speciosa* (500 mg/kg) was administered to a group of ten mice (five males and five females). We observed various behaviour parameters including convulsion, hyperactivity, sedation, grooming, loss of righting reflex, increased or decreased respiration, and food and water intake over a period of 5 days. After this period, the animals were euthanized by cervical dislocation, their stomachs were removed, an incision along the greater curvature was made, the number of ulcers (single or multiple erosion, ulcer or perforation) was counted, and hyperaemia was evaluated.

2.6. Acetic acid-induced abdominal writhing

Mice were treated according to the protocol described by Matheus et al. (2005). Briefly, the total number of writhing following intraperitoneal administration of a 2% (v/v) acetic acid solution (AA) was recorded over a period of 20 min, starting 5 min after AA injection. Mice were pre-treated with latex or vehicle 60 min before administration of AA. The positive control group was composed of animals pre-treated with ASA (200 mg/kg).

2.7. Formalin test

The procedure we used was similar to the method described by Gomes et al. (2007). The mice received an injection of 20 μl of formalin (2.5%, v/v) into the dorsal surface of the left hind paw. Immediately after injection, we recorded the time that the animal spent licking the injected paw. The nociceptive and inflammatory response developed in two phases: the first occurred 5 min after formalin injection (first phase, neurogenic pain response), and the second occurred 15–30 min after formalin injection (second phase, inflammatory pain response). The animals were pre-treated with oral doses of latex or ASA 60 min before injection of formalin.

2.8. Hot plate test

Mice were tested according to the method described by Sahley and Berntson (1979) and adapted by Matheus et al. (2005). Animals were placed on a hot plate (Insight equipments, Brazil) set at $55 \pm 1\,^{\circ}C$. Reaction times were recorded when the animals licked their fore- and hindpaws and jumped at several intervals of 30 min after the oral administration of different doses of latex. Baseline was considered as the mean of the reaction times obtained 30 and 60 min before the administration of latex and was defined as the normal reaction of the animals to the temperature. Increases in baseline (%) were calculated by the formula: ((reaction time × 100)/baseline) − 100.

2.9. Paw oedema

The method we used for inducing paw oedema was similar to the method described by Ferreira (1979). Carrageenan (1%), histamine (300 μg), bradykinin (2 μg), or serotonin (0.5 μg) were injected intraplantarly in rats 60 min after oral administration of *Hancornia speciosa* or vehicle. Rat paw oedema was measured by pletismography 1, 2, 3, and 4 h after carrageenan injection; 1 h after serotonin or histamine injection; or 30 min after bradykinin intraplantar injection. The results were converted to oedema (in μl).

2.10. Subcutaneous air pouch (SAP)

The method was similar to that described by Romano et al. (1997) with several modifications described in Raymundo et al. (2011). Air pouches were produced by subcutaneous injection of 10 ml of sterile air into the intrascapular area of the backs of the rats. After 3 days, we injected another 10 ml of air to maintain the pouches. Three days after the last injection, the animals received an injection of 0.5 ml of a sterile carrageenan suspension (1%) into the SAP. The animals were pre-treated with oral doses of latex or ASA (200 mg/kg) 60 min before injection of carrageenan. The animals were killed 4 h after carrageenan injection, and the cavity was washed with 2 ml of sterile PBS. The liquid in the SAP was collected and quantified. An aliquot of the exudate was diluted 1:20 in Turk liquid (0.5% violet crystal dissolved in 30% acetic acid). The total number of cells was determined with the aid of a haemocytometer. The exudates were centrifuged at $170 \times g$ for 10 min at 4 °C, and the supernatants were collected and stored at −20 °C until usage. The

pellets were collected and utilised as described below for enzyme expression.

2.11. IL-6, TNF-α, PGE2, and total protein measurements

Supernatants from exudates collected in the SAP were used to measure TNF-α, IL-6, protein, and PGE2. TNF-α and IL-6 quantification were done with enzyme-linked immunosorbent assays (ELISAs) using protocols supplied by the manufacturer (Peprotech). PGE2 was quantified using EIA kits (Cayman Chemical Co., MI, USA) according to the method described by Pradelles et al. (1985). The protein content of each supernatant was determined using the BCA method (BCA™ Protein Assay Kit, Pierce).

2.12. Nitrate measurement

To evaluate nitric oxide (NO) production, nitrate (the stable metabolite of NO) concentrations in the supernatants were measured according to the protocol described by Xu et al. (2000), with some modifications described in Raymundo et al. (2011). An aliquot of 100 μl of sample was transferred to a microplate and incubated with an equal volume of Griess Reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride, 10% H_3PO_4) for 10 min at room temperature (Green et al., 1982). The absorbance was measured at 540 nm using a microplate reader, and the nitrate concentration was calculated using a standard curve of sodium nitrate.

2.13. Detection of iNOS and COX2 enzyme expression

The procedure we used to detect enzyme activity was similar to the protocol described by Oliveira et al. (2006). Briefly, the cell pellet obtained after centrifugation of SAP exudates was washed with cold PBS and lysed with cold lysis buffer (10% NP40, 150 mM NaCl, 10 mM Tris HCl pH 7.6, 2 mM PMSF, and 5 μM Leupeptin). Cell debris was removed by centrifugation (12,000 × *g*, 4 °C, 10 min). After determination of protein concentration of each suspension by the BCA method (BCA™ Protein Assay Kit, Pierce), the suspensions were boiled in Laemmli buffer (100 mM DTT, 0.1% Bromophenol Blue). For SDS-PAGE, aliquots of 30 μg of protein of each sample were subjected to electrophoresis in 8% polyacrylamide gels. Following electrophoresis, the proteins were electrophoretically transferred onto nitrocellulose membranes. Membranes were blocked with 5% non-fat dried milk in Tris buffered saline with Tween (TBS-T: 10 mM Tris–HCl, 150 mM NaCl, 0.1% Tween 20) at room temperature for 2 h. After washing with the TBS-T primary antibody solution, mouse monoclonal IgG anti-iNOS or anti-COX2 was applied overnight at 4 °C at dilutions of 1:1000. Membranes were washed with TBS-T and then incubated with secondary antibody solution consisting of anti-mouse IgG antibody conjugated to horseradish peroxidase at a dilution of 1:5000 for 1 h at room temperature. The blots were washed with TBS-T, incubated with enhanced chemiluminescence (ECL) reagents and exposed to photographic film (Kodak, Brazil).

2.14. Statistical analysis

All experimental groups consisted of 6–10 mice. The results are presented as mean ± S.D. The area under the curve (AUC) was calculated with Prism Software 5.0. Statistical significance between groups was performed by applying analyses of variance (ANOVA) followed by Bonferroni's test. *P* values less than 0.05 ($P < 0.05$) were considered significant.

3. Results

After collection of latex from *Hancornia speciosa* in a 2:1 (latex:water) proportion, total protein in the solution was calculated by the Lowry method (Lowry et al., 1951). We calculated the protein concentration to be 20 mg protein/ml. For the following experiments, doses of latex were calculated based on total protein amount. Oral administration of the latex at 100 mg/kg dose did not induce any toxic effect. No behavioural alteration, lesions, or gastric bleeding was observed. Additionally, no signs of intoxication, including convulsion, death, or gastric ulcer, were observed even after 5 days of a single dose. In an attempt to calculate the lethal dose (LD_{50}), groups of mice received the latex in doses of 0.5, 1, and 1.5 g/kg. At the highest dose of latex, lethality was not observed indicating that latex was nearly nontoxic in mice up to this dose and that it was not possible to determine the LD_{50}. The phytochemical analyses of the latex indicate the presence of flavonoids and diterpenes that are under investigation to the identification (data not shown).

Pre-treatment of mice with various doses of latex, from 0.01 to 1.3 mg/kg, reduced the total contortions induced by acetic acid. With the exception of the lowest dose (0.01 mg/kg), all other doses demonstrated a significant and dose-dependent effect (Fig. 1A). The effect observed with the 0.1 mg/kg dose was similar to that obtained with the positive control drug acetylsalicylic acid (ASA) at 200 mg/kg. As the 0.01 mg/kg dose did not demonstrate a significant inhibitory effect, this dose was not used in further experiments.

Injection of formalin (2.5%) induces a biphasic licking response in the injected paw of the mice. The first phase occurred until 5 min after injection and the second phase occurred between 15 and 30 min after formalin injection. As shown in Fig. 1B, latex treatment did not reduce the time that the animal spent licking the formalin-injected paw during the first phase. However, during the second phase, latex treatment induced dose-dependent inhibition (0.1 mg/kg = 1%; 0.6 mg/kg = 38.2%; 1.3 mg/kg = 48.8%). In this model, the effect observed with the dose of 0.6 mg/kg was similar to that observed with 200 mg/kg ASA (102.3 ± 6.9* s). To investigate a possible central antinociceptive effect caused by latex treatment, the animals were pre-treated with latex, and the antinociceptive effects were evaluated using the hot plate model. None of the doses that we tested were able to increase the latency time in this model (vehicle = 11.8 ± 4.3 s; morphine = 25.3 ± 7.71 s; latex at 1.3 mg/kg = 14.3 ± 3.8 s; 3 mg/kg = 13.5 ± 4.4 s; 5 mg/kg = 13.1 ± 5.1).

The important inhibitory effects caused by latex treatment in the second phase of the formalin model (the inflammatory phase) led us to test its effects in another model of inflammation. We chose to investigate the effects of the latex on rat paw oedema induced by different phlogistic agents. Fig. 2 shows that intraplantar injection of carrageenan (1%) induced crescent oedema formation until the 4th hour after administration. Pre-treatment with latex reduced the formation of oedema. These results are better visualised by calculating the area under the curve (AUC) for each dose that we administered. A significant reduction in the AUC was observed with all three doses of latex. The dose of 1.3 mg/kg latex also inhibited oedema induced by bradykinin, histamine, and serotonin (Fig. 3). To complement the data obtained in the paw oedema model, we decided to evaluate the effects of latex in another model of inflammation, the subcutaneous air pouch model. This model involves synovial inflammation caused by subcutaneous injections of air into the back. This procedure induces the proliferation of cells that stratify on the surface. Injection of carrageenan (1%) into the mouse air pouches drastically increased the exudate volume into the pouch, up to twice the level of the mice that received PBS in the SAP. Pre-treatment of mice with latex significantly suppressed the

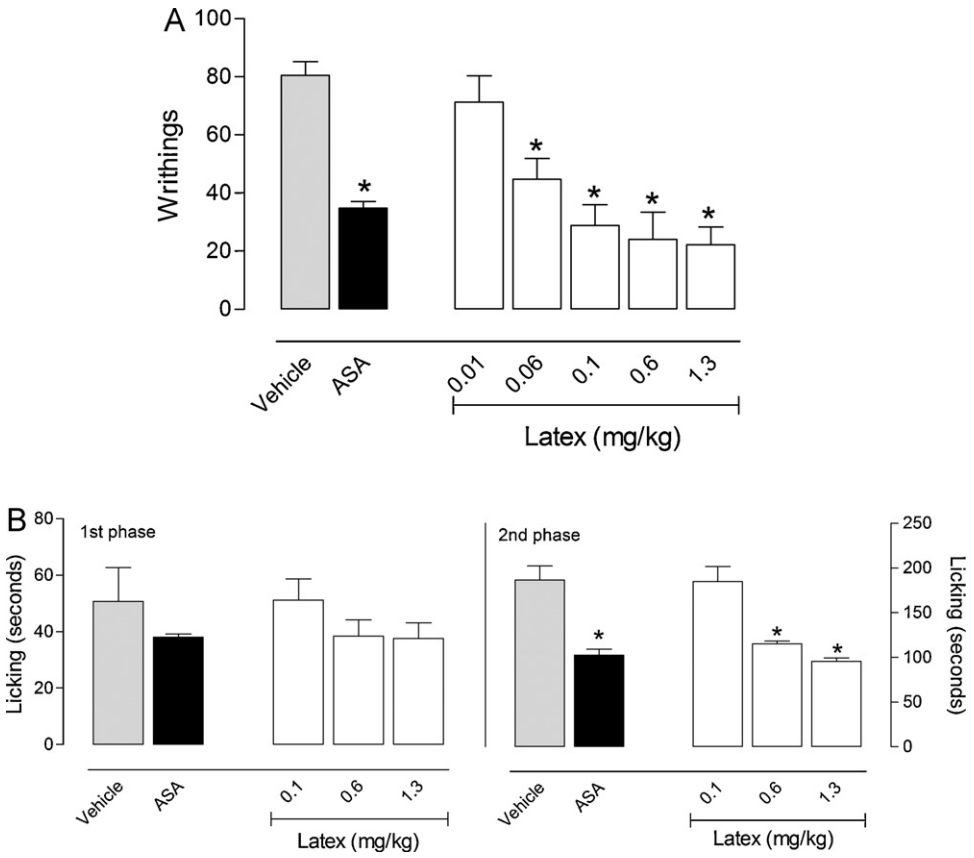

Fig. 1. Effects of the latex from *Hancornia speciosa* on acetic acid-induced abdominal writhing (A) and formalin-induced licking (B) in mice. Animals were pre-treated with oral administration of different doses of latex (open bars), acetylsalicylic acid (ASA, 200 mg/kg, black bars), or vehicle (gray bars). The results are presented as mean ± S.D. (n = 6–10) of writhing or time that the animal spent licking the formalin-injected paw. Statistical significance was calculated by ANOVA followed by Bonferroni's test. *P < 0.05 when compared to vehicle-treated mice.

volume of exudate recovered from the air pouches. Carrageenan treatment also caused a 5-fold increase in the exudate protein concentration, and the pre-treatment of mice with latex significantly suppressed the carrageenan-induced protein leakage in a dose-dependent manner. Both exudate volume and protein concentration were significantly reduced by latex doses of 0.6 and 1.3 mg/kg (Fig. 4).

In the absence of latex, white blood cells in the carrageenan-injected air pouch exudates were markedly increased, up to 20-fold greater than the control level (4.1 ± 1.1 vs. 84.6 ± 12.4). These numbers were markedly reduced by about 51.9%, 75.3%, 77.6%, and 85.1% by pre-treatment with crescent doses of latex (Fig. 5).

As shown in Fig. 6, carrageenan also dramatically increased the levels of nitric oxide (NO) and inducible nitric oxide synthase (iNOS) enzyme expression in the exudates when compared to the vehicle-treated group. A similar pattern was also observed in PGE2 and cyclooxygenase-2 (COX2) enzyme expression. Despite the fact that latex pre-treatment caused a reduction in iNOS expression, no inhibition was observed in the NO levels. We also observed that crescent doses of latex led to a reduction in both PGE2 levels and COX2 enzyme expression.

An increase in TNF-α and IL-6 levels was observed followed carrageenan injection in the SAP. Even the lowest dose of the latex (0.06 mg/kg) was able to induce a reduction in the production of IL-6 and TNF (Fig. 7).

4. Discussion

We demonstrated for the first time that latex obtained from *Hancornia speciosa* has anti-inflammatory activity. We described the mediators involved and the possible mechanisms by which the latex reduces inflammation.

It is well known that inflammatory and pain diseases remain some of the most important health problems in the world. Currently, few drugs are effective in treating these disorders, and many of them cause deleterious side effects. Therefore, the search for new therapies is extremely important.

The latex from *Hancornia speciosa* was not able to decrease the time that the animals spent licking the injected paw during the first phase after formalin injection; however, the second inflammatory phase was inhibited by latex treatment. This suggests a possible inhibition of inflammatory mediators released in the paws of the mice, and it also corroborates the inhibitory effect of the latex on the acetic acid-induced writhing response. The injection of formalin was used to test the involvement of neurogenic or inflammatory pain. Phase 1 corresponds to acute neurogenic pain that is sensitive to drugs that interact with the opioid system and is also due to interaction of inflammatory mediators with nociceptive receptors (Chapman and Dickenson, 1992; Parada et al., 2001). Phase 2 corresponds to inflammatory pain that is inhibited by nonsteroidal anti-inflammatory drugs (Hunskaar and Hole, 1987; Rosland et al., 1990).

To complement the results obtained from the second phase of the formalin-induced licking response (the inflammatory phase), latex treatment was tested in models of inflammation (rat paw oedema and subcutaneous air pouch) induced by different phlogistic agents. Acute inflammation, such as carrageenan-induced oedema, is a multi-mediator phenomenon that involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, histamine, bradykinin, leukotrienes and

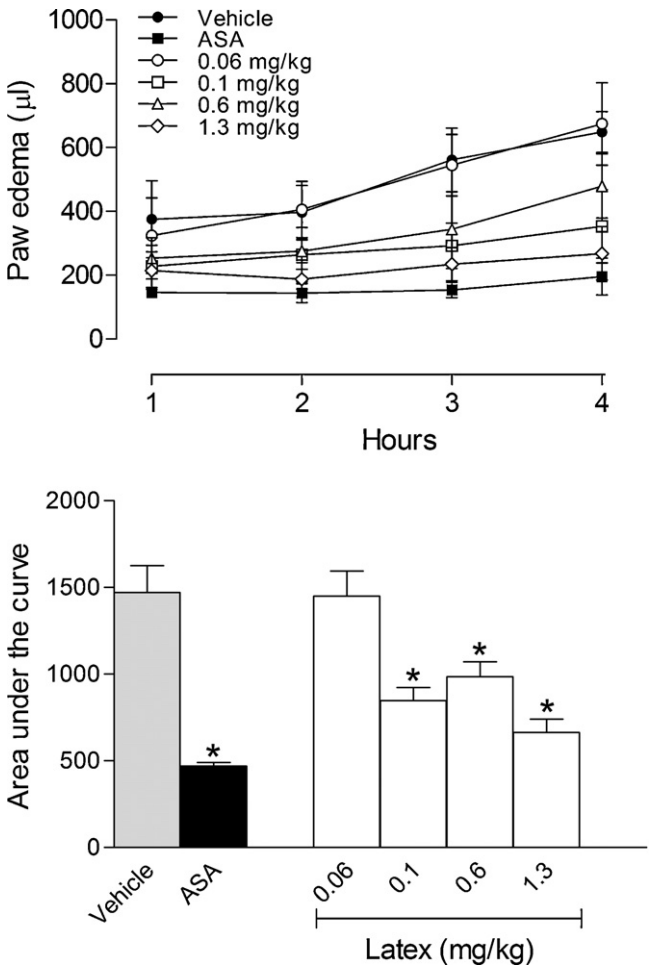

Fig. 2. Effects of the latex from *Hancornia speciosa* in the carrageenan-induced rat paw oedema model. Animals were pre-treated with oral administration of different doses of the latex. Control groups were composed of animals treated with vehicle or acetylsalicylic acid (ASA, 200 mg/kg, p.o.). The results are presented as mean ± S.D. (n = 6–10) of paw oedema (in µl, A) or area under the curve calculated by Prism Software 5.0 (in B). Statistical significance was calculated by ANOVA followed by Bonferroni's test. *P < 0.05 when comparing morphine-, *Hancornia speciosa*- or ASA-treated mice to the vehicle-treated group. Certain groups have no error bars shown because the bars are smaller than the symbol.

serotonin. The oedema formed in this model is divided into two phases. The first phase (until 2 h after carrageenan injection) is due to liberation of histamine, serotonin, and bradykinin in the paw tissue, while the second phase (3 and 4 h after carrageenan injection) is sustained by liberation of prostaglandins (Di Rosa, 1972). Preventing these mediators from reaching the injured site or from inducing their pharmacological effects will normally ameliorate inflammatory and other symptoms. Our results show that the latex of *Hancornia speciosa* possesses a significant anti-oedematogenic effect on paw oedema induced by carrageenan at all time points. These effects may be related to a reduction in the liberation of mediators (histamine, serotonin, or bradykinin) on local tissue or a blockage of the receptors that are the corresponding targets of the different mediators cited before. These results were also observed when paw oedema was induced by bradykinin, histamine or serotonin. This suggests that the anti-oedematogenic effects could be due to a direct blockage of the receptors for each mediator. Although it is well known that the first phase of formalin-induced licking response is also due to the participation of bradykinin, serotonin, and histamine activating the nociceptors (Chapman and Dickenson, 1992; De Campos et al., 1996; Parada et al., 2001), the absence of inhibitory effect from latex in this phase could be

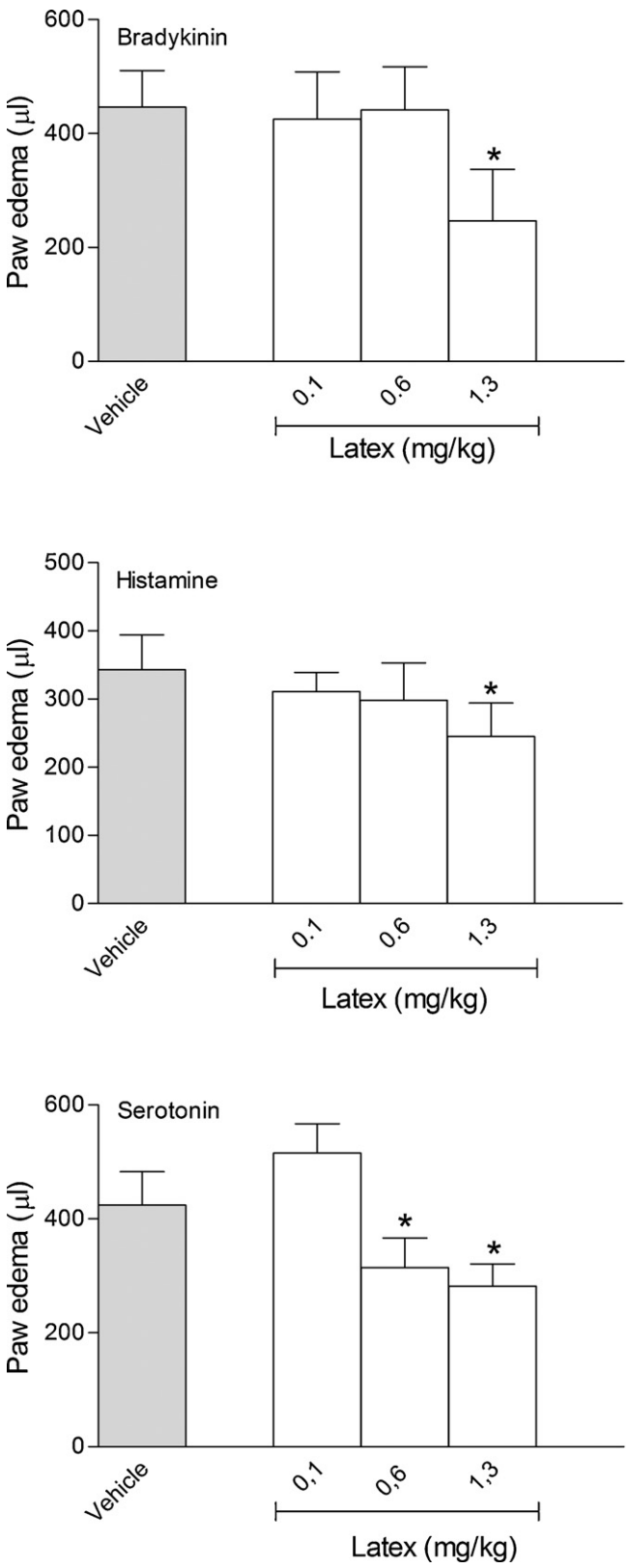

Fig. 3. Effects of the latex from *Hancornia speciosa* on bradykinin-, histamine-, or serotonin-induced rat paw oedema. Animals were pre-treated with oral administration of different doses of latex. Paw oedema was measured 30 min (bradykinin) or 1 h (histamine and serotonin) after the injections of phlogistic agents into the rat paws. The results are presented as mean ± S.D. (n = 6–10) of paw oedema (in µl). Statistical significance was calculated by ANOVA followed by Bonferroni's test. *P < 0.05 when comparing *Hancornia speciosa*-treated mice to the vehicle-treated group.

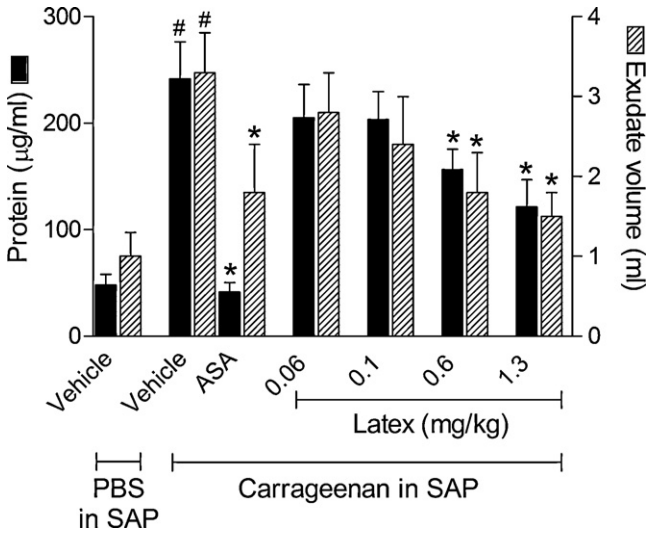

Fig. 4. Effects of the latex from *Hancornia speciosa* on the subcutaneous air pouch (SAP) model. Animals were pre-treated with oral administration of different doses of latex 1 h prior to carrageenan (1%) injection into the SAP. The results are presented as mean ± S.D. (n = 6–10) of protein (mg/ml) or exudate volume (in ml). Statistical significance was calculated by ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ when comparing latex-treated mice to the vehicle-treated group; $^{\#}P < 0.05$ when comparing vehicle-treated mice to the PBS-treated group.

explained by the hypothesis that the latex did not directly interact with nociceptors but only with the inflammatory receptors.

Using an *in vivo* air pouch inflammation model, we showed that carrageenan increased both the exudate volume and the exudate protein concentration, which indicates vascular leakage of serum contents. Among the diverse inflammatory mediators that can induce vascular permeability, it is well known that NO and PGE2 are the major factors that are involved in the pathogenesis of many inflammatory-associated diseases (Guslandi, 1998; Ritchlin et al., 2003). Therefore, the NO and PGE2 pathways are considered two of

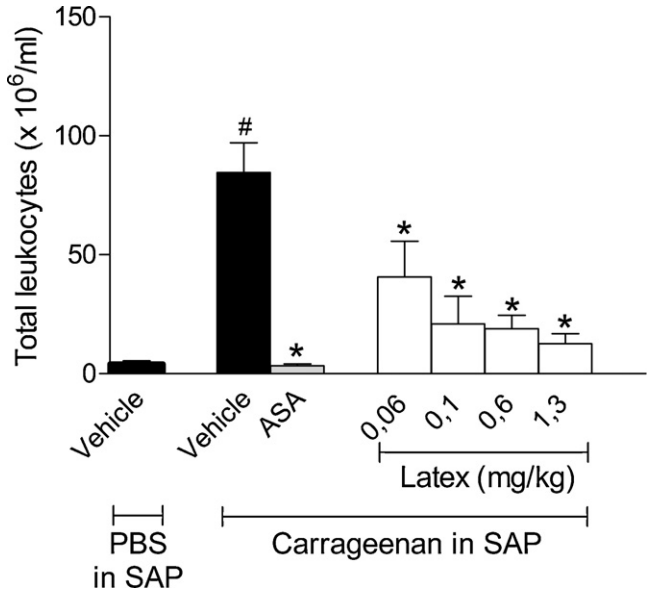

Fig. 5. Effects of the latex from *Hancornia speciosa* on leukocyte migration into the subcutaneous air pouch (SAP) model. Animals were pre-treated with oral administration of different doses of latex 1 h prior to carrageenan (1%) injection into the SAP. The results are presented as mean ± S.D. (n = 6–10) of total leukocytes ($\times 10^6$ cells/ml). Statistical significance was calculated by ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ when comparing latex-treated mice to the vehicle-treated group; $^{\#}P < 0.05$ when comparing vehicle-treated mice to the PBS-treated group.

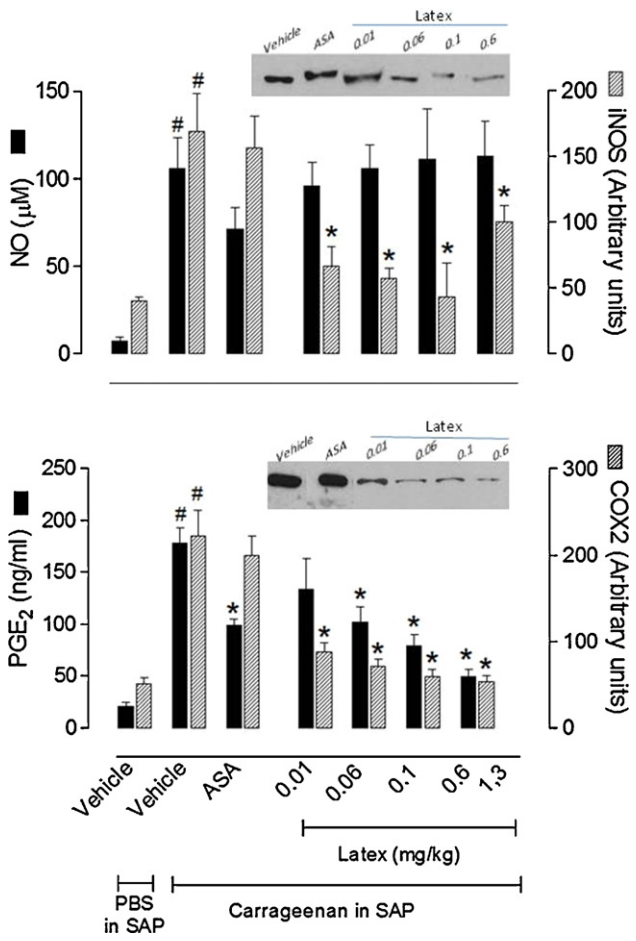

Fig. 6. Effects of the latex of *Hancornia speciosa* on nitric oxide (NO) and PGE2 production and inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX2) expression in the subcutaneous air pouch (SAP) model. Animals were pre-treated with oral administration of different doses of the latex 1 h prior to carrageenan (1%) injection into the SAP. The results are presented as mean ± S.D. (n = 6–10) of NO levels (in μM), PGE2 levels (in ng/ml), and iNOS and COX2 enzyme expression (in arbitrary units). Statistical significance was calculated by ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ when comparing latex-treated mice to the vehicle-treated group; $^{\#}P < 0.05$ when comparing vehicle-treated mice to the PBS-treated group.

the main pathways of inflammatory processes that can be blocked by inhibitors of iNOS and COX2 (Romano et al., 1997; Wallace et al., 1999). In this study, *Hancornia speciosa* reduced both exudate volume and protein concentration even at a dose of 0.6 mg/kg, suggesting a suppression of vascular leakage.

Carrageenan is also an important chemotactic agent because it induces the migration of inflammatory cells such as neutrophils and macrophages. These cells play an important role in the inflammatory process by secreting cytokines such as TNF-α and IL-6 (Oliveira de Melo et al., 2006; Zanardo et al., 2006). Pre-treatment with *Hancornia speciosa* reduced the number of cells that migrated into the carrageenan-injected air pouches. In addition to the effect on chemotaxis, *Hancornia speciosa* appeared to suppress the activation of inflammatory cells. This was confirmed by its inhibitory activity on PGE2, which is one of the major inflammatory mediators produced by these cells.

The effect of latex on TNF and IL-6 production could be due to inhibition of prostaglandin synthesis. The regulatory effect of PGE2 on cytokine has been reported in many studies (Lin et al., 2002; Noguchi et al., 2002, 2005; Jozefowski et al., 2003) and IL-6 production is differentially modulated by PG receptor agonists (Noguchi et al., 2002). It is therefore suggested that PGE2 induces variable

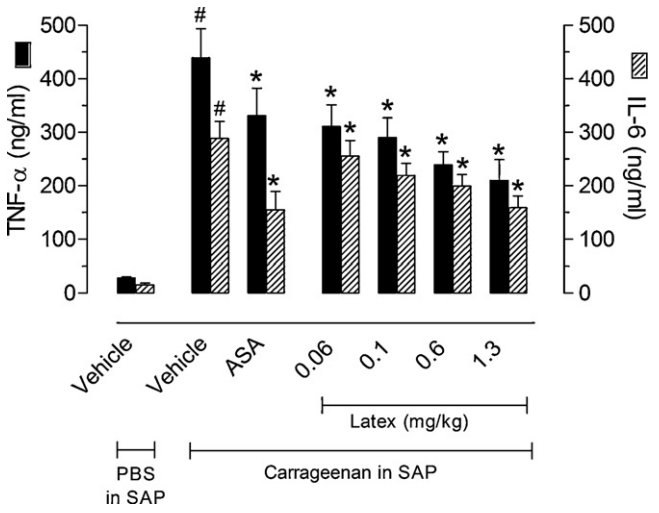

Fig. 7. Effect of the latex of *Hancornia speciosa* on TNF-α and IL-6 production in the subcutaneous air pouch (SAP) model. Animals were pre-treated with oral administration of different doses of latex 1 h prior to carrageenan (1%) injection into the SAP. The results are presented as mean ± S.D. (n = 6–10) of TNF-α (in ng/ml) and IL-6 (in ng/ml) levels. Statistical significance was calculated by ANOVA followed by Bonferroni's test. $^{*}P < 0.05$ when comparing latex-treated mice with vehicle-treated group; $^{\#}P < 0.05$ when comparing vehicle-treated mice to the PBS-treated group.

regulatory effects on cytokine production through different subtypes of receptors, the selectivity of which depends on expression of these receptors (Noguchi et al., 2002, 2005).

It is interesting to note that the inhibitory effect on PGE2 levels was accompanied by a proportional reduction in COX2 enzyme expression. In contrast, a reduction in NO levels was not observed, whereas iNOS expression was partially inhibited. One hypothesis could be that latex is reducing iNOS enzyme expression, but the enzyme activity is maintained or increased, resulting in high levels of NO.

There have been numerous conflicting papers about the role of NO in the regulation of cytokine production and enzymes expression. In some cases, NO suppresses the production of cytokines and COX2 enzyme expression, whereas in other cases, it augments or induces the expression of COX2 enzyme and cytokines production. It was showed that NO regulates NF-kB in a biphasic manner. Lower levels of NO activated NF-kB at early time-points, whereas it inhibited NF-kB activation at later time-points or with higher doses (Connelly et al., 2001).

The other explanation may be that the effect of NO depends on the type of responder cell, the cytokine, and the stimulant. For instance, NO suppresses MCP-1 but not MIP-2 production by peritoneal macrophages in response to LPS and IFN-g (Tsao et al., 1997), whereas NO augments IFN-g production by the RAW264.7 macrophage cell line in response to LPS (Jacobs and Ignarro, 2003).

Although defining the exact mechanism of action requires further study, the latex of *Hancornia speciosa* attenuated inflammatory signals by blocking the PGE2 pathway in the carrageenan air pouch inflammation model. Furthermore, *Hancornia speciosa* latex demonstrated anti-inflammatory activities in the formalin inflammation and paw oedema models. These findings confirm the popular use of the latex whose use is to drink a cup of the milk made by a mixture of water/latex (in the proportion of 1:2), several times a day (Alviano, personal observation). Our results also demonstrate the absence of toxic effect, and provide evidence that *Hancornia speciosa* might be a promising or adjunct therapy for the relief of various types of inflammation.

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